

## Antibody Trapping on Switchable Films

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**Motivation**—For Homeland Security, Sandia needs to develop compact separations systems and sensors that can selectively adsorb and detect a wide range of potential biological agents. The most sensitive and selective materials for binding bio-agents are antibodies. Unfortunately, most existing analytical systems that exploit antibodies utilize antibodies that are permanently tethered to substrate surfaces. This means that active surfaces must be discarded after only one use, and that a given active surface can only be used to detect the one species that is recognized by the specific antibody. The motivation of this research is to use Sandia's existing "protein trap" technology to reversibly adsorb and release antibody monolayers for reusable microanalytical systems.

**Accomplishment**—Sandia's reversible protein trap consists of a micro-hotplate device onto which a thermally-activated polymer (PNIPAM) film is tethered. The hotplate is used to switch PNIPAM between a room-temperature phase that repels proteins and a higher temperature phase that adsorbs proteins. The bare PNIPAM film is not particularly selective for specific proteins, adsorbing a wide range of different bio-species from solution. However, we have recently demonstrated that PNIPAM films can be made highly selective by using the thermally-activated films to grab and release monolayers consisting of antibodies. The viability of the adsorbed antibody film as a highly selective adsorbing layer for biosensors has also been demonstrated. Adsorption experiments to date have involved tobacco mosaic virus (TMV) as a model "bio-agent" and two antibodies [non-specific Rabbit immunoglobulin G (Rabbit IgG) and the TMV-specific TMV-IgG]. Component adsorption on thermally-activated PNIPAM films has been monitored using ellipsometry

(Fig. 1) and neutron reflectivity measurements performed using the LANSCE neutron scattering facilities at Los Alamos. First, the ellipsometry results show that PNIPAM films can be programmed to reversibly adsorb and release antibodies (both Rabbit IgG and the TMV-IgG). Second, TMV adsorption experiments show that TMV-IgG remains active and is highly selective for TMV when adsorbed on the PNIPAM surface. While slight TMV adsorption is observed on bare PNIPAM, no adsorption is observed when the PNIPAM is covered with Rabbit IgG, while extensive adsorption is observed when the TMV-IgG is present. Recent experiments suggest that once the TMV is bound to TMV-IgG on the PNIPAM, the antibody-antigen complex can be desorbed almost completely by cooling the PNIPAM to room temperature.

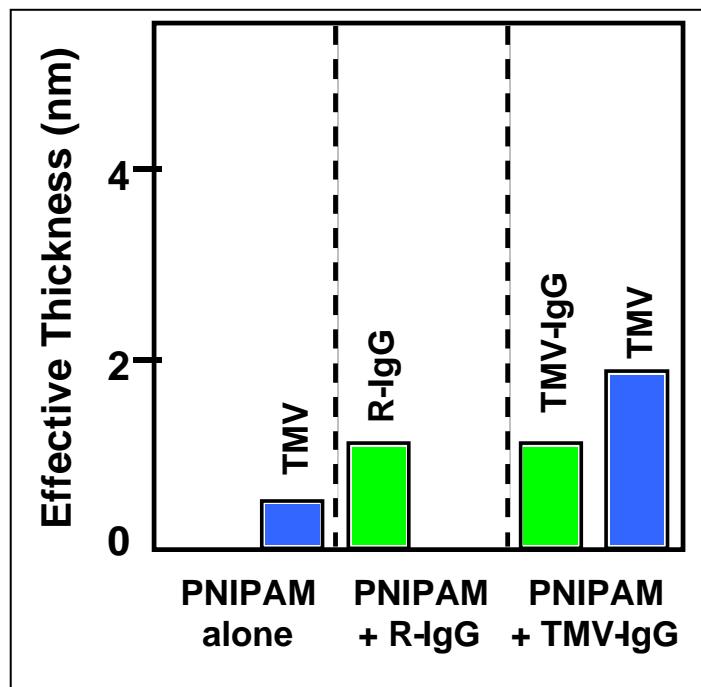
**Significance**—Now that reversible trapping of antibodies and antigens has been demonstrated, we are in a position to start learning how to integrate reversible antibody trapping into separation and sensor systems. The scheme shown in Fig. 2 illustrates how the antibody trap can be used to selectively remove specific bioagents from a feed stream in a microfluidic system. For biosensors, agents pre-concentrated and released from the trap could be sent to down-stream components, eliminating the undesired background associated with other biomaterials present in the feed stream. Alternatively, the film used for capture and release could be incorporated into an active sensor element, producing a compact device in which capture and sensing are performed simultaneously. Work is in progress to integrate the antibody trap into a shear-horizontal acoustic wave device to test this concept.

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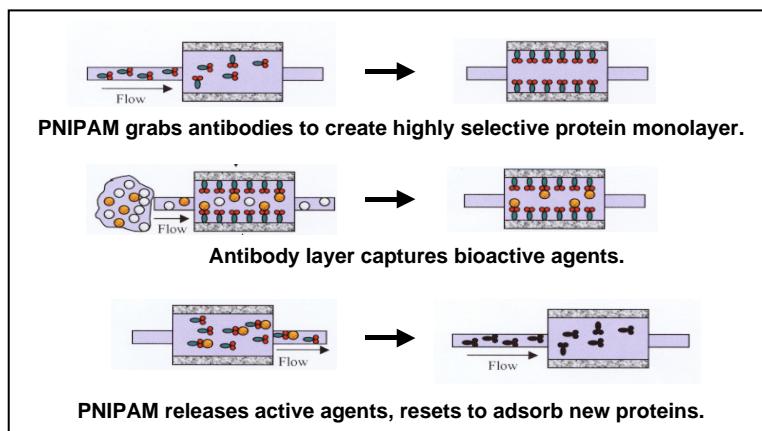
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**Figure 1.** Left – Adsorption of tobacco mosaic virus (TMV) on activated PNIPAM film. Center – Adsorption of Rabbit immunoglobulin G (R-IgG) and TMV on the R-IgG layer. Right – Adsorption of TMV-IgG and TMV on the adsorbed TMW-IgG showing the high specificity for TMV adsorption on the TMV antibody relative to the rabbit antibody.



**Figure 2.** Schematic showing the sequence of steps needed to capture and release bio-species using reversible antibody trapping.